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Phytochemical studies and HPTLC fingerprint profiling of different extracts of *Portulaca Quadrifida* L.

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Abstract---The present study was designed to detect the class of compound by phytochemical evaluation and perform the high performance thin layer chromatography (HPTLC) fingerprint analysis of hexane extract of *P. quadrifida* (HEPQ), chloroform extract of *P. quadrifida* (CEPQ), ethyl acetate extract of *P. quadrifida* (EAEPQ) and ethanol extract of *P. quadrifida* (EEPQ). The successive extraction based on elutropic solvent system was utilised for extraction. The preliminary phytochemical screening was carried out for all the extract. For the HPTLC fingerprint profiling the precoated Merck silica plates were used as stationary phase while, mobile phase utilised was Toluene: Chloroform: Ethanol (5:4:1 v/v/v). The scanning was done at White light, 254 nm and 366 nm. The presence of steroids, coumarins, flavonoids, glycosides, saponins, coumarins was found in all 4 extracts, except the terpenoids which were present in HEPQ and EAEPQ. The fingerprinting profile was successfully obtained for all four extracts of *P. quadrifida*. This profiling data can thus be utilized for quality control of the *P. quadrifida* both on qualitative and quantitative basis.

Keywords---*Portulaca quadrifida*, HPTLC, fingerprinting.

Introduction

The traditional medicines are still believed to be one of the most effective and promising source for treatment and diagnosis of many ailments. The herbal or plant source have complex composition which attribute to their multifaceted

pharmacological activities (1), (2). The herbs or medicinal plants have not only gained the importance in health care system in form of the herbal or polyherbal formulation, but it has also served as major source for plant derived compounds (3). The different geographical location, growing and storage conditions do affect the chemical components of the medicinal plants. With growing need of these medicinal plants, it is equally important to monitor the standard of the compounds present in it and hence quality control is to be done periodically. The presence of complex nature of the chemical constituents is hence to be analyzed qualitatively or quantitatively. The simple phytochemical studies help in determining the class of compounds present in particular extract of plant. The constituent presence can be analyzed by different analytical techniques. The sophisticated analytical techniques deal with the comprehensive and integrative way with the compounded nature of the plant bioactive (4), (5), (6). One such sophisticated technique which has a major contribution in standardization i.e., quality analysis per se is high performance thin layer chromatography (HPTLC). The HPTLC is basically working on the principles of thin layer chromatography and has full capabilities of separating the multiple constituents in any medicinal plant. Thus, it helps in achieving a typical profile which remains unique for particular extract of specific plant which is termed as fingerprint profiling (7), (8),

Portulaca quadrifida L. commonly known as chicken weed belonging to the family Portulacaceae are majorly found in the tropical regions of India. It is a small diffused and succulent herb with a life cycle that lasts only for a year. They are widely grown on bare patches and among rocks, on sandy or stony soils, from sea-level up to 2000 m altitude. The plant is tolerant and adaptive to wide range of soil types, but the herb is best grown in sandy loams (9). The plant is commonly known as Chicken Weed in English; Baralunia or Chota Luniya or Lonika in Hindi; Bhummy Gol or Kathe Chanval in Marathi (10). The herb is useful in treatment of disease such as neurological disease, convulsion, inflammation, dysentery, gastrointestinal disorders and antioxidant. The pharmacological activities reported are anticancer, antinociceptive, anticonvulsant, antifungal, antidiarrheal, anti-inflammatory and antioxidant activity ^{11, 12}, (13), (14). Hence this plant was selected to study the phytochemical screening and HPTLC fingerprint profiling. Thus, this analysis data can be utilized as a parameter in evaluating the quality of the plant *P. quadrifida* as well as the source to identify, isolate and study a particular class of compound from it.

Experimental

Plant procurement and authentication

The whole weed *Portulaca quadrifida* L. (Family: Portulacaceae) was collected from Satpuda region. The plant material was authenticated by Dr. Rajendra Shinde, Head, Department of Botany and Director, Blatter Herbarium, St. Xavier's College, Mumbai 400 001. The fresh plant specimen was identified as *Portulaca quadrifida* L. belonging to family Portulacaceae, with Herbarium specimen number 16926 of H. Santapau; dated 06/08/2018.

Drying of plant material

The whole fresh plant material was washed, and shade dried at temperature not exceeding 40 °C. The dried material was subjected to mechanical grinding to obtain the coarse powder (# 40-80) and was stored in airtight container till further use.

Plant extraction

The extraction was done using eluotropic solvent system using the solvents from non-polar to polar solvents. The solvents selected were hexane, chloroform, ethyl acetate and ethanol. The Soxhlet extraction method was used. The dried plant material was subjected to successive extraction using eluotropic solvent system to obtain the following extracts i.e., hexane extract of *P. quadrifida* (HEPQ), chloroform extract of *P. quadrifida* (CEPQ), ethyl acetate extract of *P. quadrifida* (EAEPQ) and ethanol extract of *P. quadrifida* (EEPQ). The obtained fractions were transferred into airtight container and stored in desiccator at room temperature until further use (15).

Preliminary phytochemical studies

The obtained extracts of *P. quadrifida* i.e., HEPQ, CEPQ, EAEPQ and EEPQ were subjected to phytochemical screening for detection of alkaloids, flavonoids, steroids, anthraquinones, glycosides, tannins, essential oil, saponins, terpenoids, coumarins and phenols using standard procedures to identify them (16), (17).

Fingerprint profiling (8), (18)

The HPTLC fingerprinting was carried out with the mobile phase as Toluene: Chloroform: Ethanol (5:4:1 v/v/v). The 10 mg of plant sample was dissolved in 1ml of chromatographic grade methanol to prepare the sample. The stationary phase was Merck, HPTLC Silica gel 60 F₂₅₄ plate with 200 x 100 mm dimensions. The mobile phase selected was toluene: chloroform: ethanol in the ratio of 5:4:1 v/v/v and the solvent run were 70 mm. With the Linomat 5 sample applicator of HPTLC system by CAMAG the sample volume of 5 and 10 µL was applied in the form of 8mm band on to the HPTLC plate. The chromatogram was developed in twin trough glass chamber 20 x 10 saturated with the 20 ml of mobile phase system for total of 20 min. the plates were allowed to air dry. The densitometer TLC scanner 4 was used to scan the plate at white light, at 254 nm and 366 nm.

Results and Discussion

Preliminary phytochemical studies

The phytochemical screening of 4 different extracts of *P. quadrifida* for alkaloids, flavonoids, steroids, anthraquinones, glycosides, tannins, essential oil, saponins, terpenoids, phenol and coumarins are depicted in detail below in the table number 1. The phytoconstituents found present in HEPQ and EAEPQ are flavonoids, steroids, glycosides, saponins, terpenoids and coumarins. While the

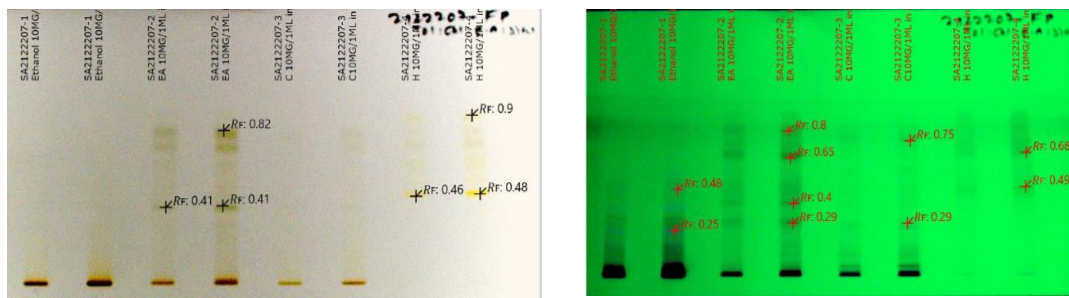
constituents found in CEPQ and EEPQ are flavonoids, steroids, glycosides, saponins, and coumarins.

Table Number 1: Phytochemical constituents of different extracts of *P. quadrifida*

Sr. No.	Class of Compound	Reagent Used	Result			
			HEP Q	CEP Q	EAEP Q	EEP Q
1	Alkaloids	Dragendroff's reagent	-	-	-	-
2	Flavonoids	Natural product reagent	+	+	+	+
3	Steroids	Lieberman-Burchard reaction	+	+	+	+
4	Anthraquinones	Bontrager's test	-	-	-	-
5	Glycosides	Keller-Kalani test	+	+	+	+
6	Tannins	Alcoholic Ferric chloride	-	-	-	-
7	Essential oil	Vanillin reagent	-	-	-	-
8	Saponins	Foam test	+	+	+	+
9	Terpenoids	Salkowski test	+	-	+	-
10	Phenol	Alcoholic Ferric chloride	-	-	-	-
11	Coumarins	Ammonia test	+	+	+	+

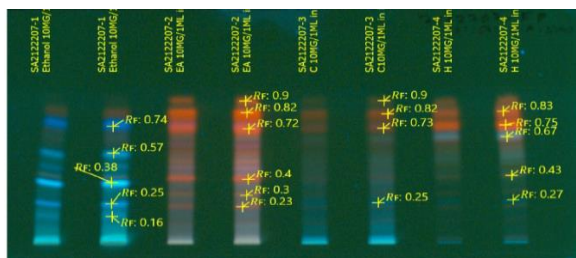
Fingerprint profiling

The evaluation of thin layer chromatography is one of the widely accepted method for standardizing the content in medicinal plant extracts. The images of chromatographic plate indicating the fingerprint profiling of HEPQ, CEPQ, EAEPQ and EEPQ is as indicated in figure number 1. Briefly in the chromatographic plates the track number 1 and 2 is of EEPQ, track number 3 and 4 of EAEPQ, track number 5 and 6 of CEPQ and finally track number 7 and 8 of HEPQ. All the odd track numbers were loaded with 5 μ l and even track numbers with 10 μ l of respective extracts. The images are recorded under white light, UV short wave i.e., 254 nm and UV long wave i.e., 366nm (Fluorescence). The maximum compounds are reported in fluorescence i.e. at 366nm followed by 254 nm compared to under white light.



1a: Image after development under white light

1b: Image after development under 254nm



1c: Image after development under 366 nm

Figure number 1: Images of chromatographic plate of different extract of *P. quadrifida* development under white light, 254nm and 366 nm.

The thin layer chromatographic method is most promising, as a virtue of good sensitivity, effective separation of various constituents in plant extract, simplicity and easy operation in comparison to other analytical method (19). The HPTLC can determine the constituents accurately and at a very low concentration hence the plant material required is often very less (6), (7), (8). The polarity wise separation of the compounds can be seen in this method and hence predicting its application in isolation of particular compound. With the growing need for standardization of plant extract the HPTLC has proven to be the efficient and reliable method. The fingerprint profile is a stable backup for comparison of constituents in different extract of same plant or same extract of different species of same plant (7), (8).

Conclusion

The study concludes the detection and presence of various active molecules in different extracts of the *P. quadrifida* which was successfully achieved by the preliminary phytochemical studies followed by fingerprint profiling. Thus, this data can be used to further extrapolate to isolate and study the constituents which can have potential pharmacological activity and also to standardize *P. quadrifida*.

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